Digestibility by Lambs of Water-Stressed Alfalfa as Determined by Total Collection or Internal Markers[1]

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ABSTRACT

A lamb digestion trial was conducted to compare the ability of internal markers to predict digestibility of alfalfa. Lambs were fed alfalfa hay grown with varying amounts of water stress where water per harvest ranged from 10 to 20 cm/ha and yield ranged 1400 to 4200 kg/ha. In vivo dry matter digestibility was most highly correlated with digestibility determined using acid detergent fiber insoluble ash (r = .80) followed by acid insoluble ash (r = .69). In vivo digestibility of the fibrous components was most highly correlated with digestibility determined by acid detergent insoluble ash and indigestible neutral detergent fiber followed by acid lignin and acid insoluble ash.

INTRODUCTION

Due to the relative difficulty and expense of conducting conventional digestion trials, the use of inert markers to predict digestibility of diets, especially forages, has received considerable attention. Much of this attention has been directed toward the use of external markers that are either given orally or are infused into the rumen. The use of reliable internal markers that occur naturally in the diet has advantages over the use of external markers. Several internal markers have been evaluated with varying success. Among these are acid insoluble ash (AIA) (11), lignin (2, 8), acid detergent fiber insoluble ash (ADFIA) (9), indigestible neutral detergent fiber (INDF), and indigestible acid detergent fiber (IADF) (15). The composition of the diet fed and source of the internal marker affect the accuracy of digestibilities determined by each of these internal markers (9). Although lignin, AIA, and ADFIA have all been reported to be of value as internal markers, no information is available on the effect of variability of the environmental growing conditions of the forage on the accuracy of these markers.

The objective was to determine the ability of AIA, acid-lignin, permanganate-lignin, ADFIA, INDF, and IADF to predict digestibility of alfalfa grown with water stress.

MATERIALS AND METHODS

Alfalfa was harvested at 10% bloom from plots having water application of 20 (low stress), 15 (medium stress), and 10 (high stress) cm/ha per harvest in 1984. Four varieties (Vangard, Cody, Zia, and Dawson) were harvested with a flail chopper from the fourth and fifth harvests and air-dried in a greenhouse. Forage from the different varieties and harvests were combined by stresses.

In vivo digestibility determinations were made as described previously (10). Twelve Suffolk and Hampshire crossbred lambs averaging 40 kg were randomly allotted to one of the three alfalfa diets. All lambs were fed the same alfalfa diet for 7 d and were then fed their assigned diet at 1% of body weight for 10 d. Water and trace-mineralized salt were provided ad libitum. Hay and salt provided sufficient energy, protein, and nutrients to meet or exceed the maintenance requirements of sheep (5). Lambs were housed in individual pens with

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slotted floors and were equipped with fecal collection bags. Feces were collected, weighed, and frozen daily during the last 4 d of the trial.

Feces from each lamb were composited and subsampled at the end of the collection period. About 500 g of feces were dried at 65°C and ground through a 1-mm screen in a Wiley mill. Representative forage samples were collected daily. Forage samples were composited and subsampled at the end of the collection period.

Forage and feces were analyzed for DM by drying at 65°C to minimize losses of organic compounds (3). Organic matter (OM) was determined by ashing at 450°C for 4 h in a muffle furnace (3). Acid detergent fiber, cell wall constituents (NDF), and cellulose [ADF–permanganate lignin (PL)] were determined by the procedures of Goering and Van Soest (3). Hemicellulose was calculated as the difference between NDF and ADF. Lignin was determined by the 72% sulfuric acid procedure (AL) of Van Soest (12) and by the permanganate procedure (PL) of Van Soest and Wine (13). Acid detergent fiber insoluble ash was the residual ash (450°C) following ADF determination.

Total apparent digestibilities of DM, OM, NDF, ADF, hemicellulose, and cellulose were determined by seven methods: 1) total collection, 2) AL ratio, 3) PL ratio, 4) AIA ratio, 5) ADFIA ratio, 6) IADF ratio, and 7) INDF ratio. Determinations of AL, PL, and ADFIA on both hay and feces were made as described previously. Acid insoluble ash was determined by the 2 N HCl procedure of Van Keulen and Young (11). The INDF and IADF were determined by incubation of 300 mg of plant or fecal material in rumen fluid and buffer for 96 h, followed by 2 ml 6 N HCl and 100 mg pepsin for 48 h, refluxing in either ADF or NDF solution for 1 h, and filtering (15).

Internal marker data were statistically analyzed by analysis of variance as a 3 × 7 factorial using the general linear models procedure of the Statistical Analysis System (7).

RESULTS AND DISCUSSION

The composition of the alfalfa is presented in Table 1. Results were similar to those reported in a previous intensive agronomic study (6). Because much alfalfa is subjected to periodic water stress, these treatments might be representative of some of the environmental variations that can occur during alfalfa growth. Water stress caused in vivo DM digestibility to decrease from 60.9 to 56.7% (Table 3) as described previously (10). Therefore, the following results pertain to utility of several internal markers on forage grown under differing environments.

The recovery of AL, PL, AIA, ADFIA, IADF, and INDF by total fecal collection from the rations is in Table 2. Recovery of AL, IADF, and INDF averaged 107.6, 101.1, and

TABLE 1. Fiber and nutrient composition of alfalfa at different water stresses.

Item	Water stress		
	Low	Medium	High
Dry matter, %	89.2	89.5	89.4
		(%, dry matter basis)	
Organic matter	83.0	83.5	76.6
Ash	17.0	16.5	23.4
NDF	45.5	41.4	37.8
ADF	32.8	29.7	30.3
Hemicellulose	12.8	11.3	7.4
Cellulose	16.6	15.0	11.5
Acid lignin	6.8	7.0	6.3
Permanganate lignin	12.6	11.7	9.2
ADF insoluble ash	4.5	4.1	9.2
Acid insoluble ash	3.7	3.6	9.8
Indigestible NDF	27.9	26.8	30.4
Indigestible ADF	20.2	19.1	23.5

TABLE 2. Recovery of internal markers in alfalfa grown at three water stresses determined by total fecal collection.

Item[1]	Water stress			Mean	SE[2]
	Low	Medium	High		
	(% of intake)				
AL	106.5	104.0	112.3	107.6	3.38
PL	70.3[a]	75.3[a]	91.9[b]	79.2	2.22
AIA	117.3[a]	97.2[b]	89.7[b]	101.4	3.98
ADFIA	114.9[a]	110.9[a]	89.7[b]	105.2	2.48
IADF	100.4	104.5	98.4	101.1	2.74
INDF	84.7	85.8	87.5	86.0	3.51

[a,b]Means in each row with different superscripts differ ($P<.05$).

[1] AL = Acid lignin; PL = permanganate lignin; AIA = acid insoluble ash; ADFIA = ADF insoluble ash; IADF = indigestible ADF; INDF = indigestible NDF.

[2] n = 4.

86%, respectively, and did not vary ($P>.10$) among the diets grown at the three stresses. The recovery of PL was lower than that of the other markers and increased ($P<.05$) with increased water stress. Recovery of AIA and ADFIA declined ($P<.05$) with increased water stress.

Permanganate lignin was the poorest marker for prediction of digestibility. Recovery of PL was always less than 100%. Low recovery of PL would be associated with an apparent digestibility of PL as has been reported by the others (2, 14). Apparent digestibility of the PL resulted both in an underestimation of forage digestibility and a differing trend of digestibility changes across the water stresses as compared with in vivo data (Tables 3 and 4). Wallace and Van Dyne (14) similarly reported that varying degrees of lignin digestibility may occur, which would result in invalid digestibility data. The reports of PL being a good marker have generally been when the diets consisted of grasses (2). Because of the differences in lignin structure between grasses and legumes, it may be that PL is a better marker in grasses than in legumes.

Acid lignin resulted in estimates that tended to overestimate DM digestion while a correct trend in digestibility was predicted. Fewer differences occurred between the estimates and in vivo values than when PL was used. Scales et al. (8) similarly noted that AL provided better estimates of digestibility than PL in native range grass species.

When averaged over all stresses, AIA tended to be an accurate predictor of DM and fiber digestibility; however, AIA tended to overestimate digestibility of low stress alfalfa and underestimate digestibility of high stress alfalfa. However, ADFIA accurately reflected the stress and was most highly correlated with DM digestibility. Sherrod et al. (9) reported that both AIA and ADFIA resulted in accurate estimates of digestibility if the components were above 3%. However, he sampled only one alfalfa sample and it contained .72% AIA. It may be that species are also important and that AIA is a poorer marker in alfalfa than grasses. Both IADF and INDF had lower correlations with in vivo DM digestibility than ADFIA and have the lowest correlations with in vivo OM digestibility. Indigestible NDF consistently underestimated digestibility of all fractions. Cochran et al. (1) similarly noted that the relationship between in vivo DM digestibility and digestibility determined by IADF and INDF was highly variable.

None of the internal markers were consistently accurate predictors of OM digestibility (Tables 3 and 4). However, several markers resulted in accurate estimates of cellulose digestibility. The most accurate indicator of the digestibility of the cellulose was ADFIA. In all cases, this marker resulted in the highest correlation and least mean difference between predicted digestibility and in vivo total collection.

It is anticipated that similar results would

TABLE 3. Apparent digestibility coefficients of alfalfa grown at three water stresses as calculated by total fecal collection and internal marker techniques.

Method[1]	Water stress			Mean	SE[2]
	Low	Medium	High		
	(%)				
Dry matter					
In vivo	60.9[a]	61.0[a]	56.7[b]	59.5[e]	.87
AL	63.2[a]	62.5[ab]	61.4[b]	62.4[d]	.50
PL	44.1[e]	48.2[b]	52.9[a]	48.4[b]	1.13
AIA	66.6[a]	59.3[b]	51.7[c]	59.2[b]	1.91
ADFIA	63.7[a]	61.2[a]	56.2[b]	60.4[de]	1.16
IADF	61.3[a]	62.7[a]	55.9[b]	60.0[de]	.62
INDF	54.4[a]	54.6[a]	50.5[b]	53.2[d]	.50
Organic matter					
In vivo	65.0	64.6	63.2	64.3[e]	.93
AL	67.1	65.9	67.3	66.8[d]	.28
PL	50.2[a]	52.9[a]	60.0[b]	54.4[g]	1.03
AIA	70.1[a]	63.0[b]	59.0[a]	64.0[e]	1.87
ADFIA	67.6[a]	64.7[ab]	62.8[b]	65.0[de]	1.18
IADF	65.5[a]	66.1[a]	62.6[b]	64.7[e]	.44
INDF	59.3	58.7	58.0	58.7[f]	.39
Cellulose					
In vivo	57.5[a]	55.1[ab]	50.6[b]	54.4[d]	2.00
AL	60.1[a]	56.8[b]	56.0[b]	57.7[d]	.94
PL	29.9	28.6	34.9	31.2[f]	3.04
AIA	63.7[a]	53.1[b]	44.8[b]	53.9[d]	2.96
ADFIA	60.6[a]	55.2[ab]	50.1[b]	55.3[d]	2.13
IADF	58.0[a]	57.0[a]	49.8[b]	54.9[d]	1.01
INDF	50.6[a]	47.7[ab]	43.6[b]	47.3[e]	1.23

[a,b,c]Means in each row with different superscripts differ ($P<.05$).

[d,e,f,g]Means in each column within each measurement with different superscripts differ ($P<.05$).

[1] In vivo = Total collection; AL = acid lignin; PL = permanganate lignin; AIA = acid insoluble ash; ADFIA = ADF insoluble ash; IADF = indigestible ADF; INDF = indigestible NDF.

[2] n = 4.

TABLE 4. Correlation coefficients between digestibility coefficients determined by total fecal collection and internal marker techniques (n = 12).

Item	Internal marker[1]					
	AL	PL	AIA	ADFIA	IADF	INDF
Dry matter	.22	−.62*	.69*	.80*	.63*	.65*
Organic matter	−.55	−.58*	.47	.48	.22	.35
NDF	.92**	.43	.93**	.96**	.92**	.95**
ADF	.78**	−.43	.85**	.91**	.82**	.88**
Hemicellulose	.99**	.95**	.98**	.99**	.99**	.99**
Cellulose	.82**	.04	.81**	.90**	.80**	.91**

[1] AL = Acid lignin; PL = permanganate lignin; AIA = acid insoluble ash; ADFIA = ADF insoluble ash; IADF = indigestible ADF; INDF = indigestible NDF.

*$P<.05$.

**$P<.01$.

occur in cattle because relative difference in feed value between herbages are similar for sheep and cattle. For high quality forages, having DM digestibilities above 55 to 60%, the differences are usually small (1 to 3%). In lower quality forages, the in vivo digestibility for cattle would be expected to be higher than for sheep (4).

In summary, for alfalfa hay grown under three water stresses, ADFIA was the most highly correlated of the internal markers examined with in vivo DM digestibility and most accurate indicator of in vivo fiber digestibilities.

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